Milk Proteins

By THOMAS L. McMEEKIN AND B. DAVID POLIS

Eastern Regional Research Laboratory, * *Philadelphia, Pennsylvania*

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I. Introduction

The importance of milk as a food and its availability have made milk proteins a favorite subject for investigation. This has been particularly true of casein, which for a long time was considered a pure protein (46). Primarily because it is so easily obtained, cow's milk has been investigated more frequently than any other milk. Unless otherwise stated in this review, the discussion refers to the proteins from cow's milk. Information on human milk proteins is included when the data are available. Although in recent years there have been notable advances in techniques for separating and investigating proteins, the new results have largely confirmed and extended the thorough investigations of Crowther and Raistrick (25), of Howe (58), and of Wells and Osborne (142) with regard to the individual proteins of milk and their relationship to the proteins of blood serum.

II. Protein Distribution in Milk

The amount and type of protein in milk have been estimated by several supplementary procedures. The method of determining nitrogen distribution on the basis of the amount of protein separated by isoelectric precipitation, salt fractionation, and heat coagulation is relatively simple and has given comparative information concerning the proteins in milk (8,89,109). Results obtained by this method (Table I) show, for example, that casein accounts for about 80% of the protein nitrogen of cow's milk and only 30% of the protein nitrogen of human milk. The electrophoretic method of determining the protein composition of milk gives more accurate information concerning the number and relative amounts of the components than does the nitrogen distribution method.

Since approximately 80% of the protein of milk is casein, and casein is electrophoretically heterogeneous (see casein section), it is advantageous to separate the casein from whey by acidification before determining the protein composition of milk by the electrophoretic method. The protein components of whey have been determined by Smith (118), and by Deutsch (28) by the electrophoretic method. Smith's data on the number of components of whey and their concentrations and mobilities are given in Table II.

A further method for evaluating the protein composition of milk is determining size distribution by means of the ultracentrifuge. Pedersen (99) has found the casein fraction to be heterogeneous with respect to size. Milk serum or whey contained three main boundaries. The α-component with sedimentation constant $S_{20} = 1.8$ he identified as

TABLE I

Nitrogen Distribution in Milk

Nitrogen	Cow's milk (89)		Human milk (8)	
	Mg./100 ml.	% of total N	Mg./100 ml.	% of total N
Total	540	...	162	...
Nonprotein[a]	30	5.5	36	22
Casein[b]	430	79.5	49	30
Whey protein[c]				
(total)	80	15.0	77	48
Globulin[d]	19	3.5	...	...
Albumin[e]	43	8.0	...	...
Protcose[f]	18	3.0	...	...

[a] Not precipitated by 15% trichloroacetic acid.

[b] Precipitated by acetic acid, pH 4.7.

[c] Nitrogen value of the casein filtrate (whey) minus nonprotein nitrogen.

[d] Precipitated from casein filtrate by saturation with magnesium sulfate, pH 7.0.

[e] Obtained by subtracting globulin nitrogen plus nonprotein nitrogen from the total nitrogen of the casein filtrate.

[f] Nitrogen content of filtrate from heat-coagulated milk minus nonprotein nitrogen.

TABLE II

Composition of Whey from Cow's Milk as Shown by Electrophoresis[a]

Component	Conc., %	Mobility $\times 10^{-5}$
(a) Euglobulin[b]	6	−1.7
(b) Pseudoglobulin[c]	4	−2.5
(c) Component	18	−3.6
(d) Component	12	−4.5
(e) β-Lactoglobulin	55	−5.1
(f) Component	5	−6.4

[a] Determined in Veronal buffer, pH 8.4, ionic strength 0.1, with protein concentration 1.2%.

[b] Insoluble in 0.3 saturation with ammonium sulfate and insoluble in water at isoelectric point in absence of salt.

[c] Insoluble in 0.3 saturation with ammonium sulfate but soluble in water at isoelectric point in absence of salt.

Kekwick's lactalbumin; the β-component with $S_{20} = 3.0$, as Palmer's β-lactoglobulin; and the γ-component with $S_{20} = 7.0$, as classical globulin.

III. Separation and Properties of Milk Proteins

Analytical methods are useful in revealing the amount and the approximate type of protein in milk and are valuable in following chemical

separations. For complete separation and characterization of individual proteins of milk, however, it is necessary to fractionate by chemical or other means.

1. Casein

The ease of separating casein from the other constituents of milk by acidifying to pH 4.7 has made it one of the most extensively studied proteins. Usually casein is further purified by dissolving it in milk alkali and reprecipitating at pH 4.7 one or more times before characterization or fractionation. Since casein is a mixture of proteins, is labile to alkali (22), and contains a proteolytic enzyme (141), the product obtained is somewhat determined by the method of purification. However, the electrophoretic patterns obtained by Warner (140) on reprecipitated casein were similar to the patterns reported by Mellander (87) on casein which had not been reprecipitated.

a. Comparison of Human and Cow's Casein. Mellander (87) has recently published an investigation and review of the chemical differences between casein from human milk and cow's milk. Human milk yielded only from 0.3 to 0.6% casein, as compared with 3.0 to 3.5% casein from cow's milk. Human casein was much more difficult to separate than cow's casein. In fact, the wide differences found in the sulfur content of human casein by different methods indicate that the separation of casein from the other proteins of human milk is uncertain. Mellander found that both human and cow's casein contained three components, as shown by electrophoresis. Human α-casein had a mobility of only 4.5×10^{-5} as compared with 7.4×10^{-5} for cow's α-casein in phosphate buffer of ionic strength 0.15 and pH 7.6. The greater mobility of cow's α-casein may be associated with its higher phosphorus content. Caseins from human milk and cow's milk differ markedly in the influence of pH on the electrophoretic pattern. Thus in cow's casein the electrophoretic pattern showed only one component at a pH acid to the isoelectric point, whereas the human casein electrophoretic pattern showed much less change in the distribution of components with change in pH.

b. Heterogeneity of Casein. The solubility studies of Linderstrøm-Lang and Kodama (76) demonstrated that casein is a mixture. These results have led to numerous attempts to fractionate casein into its components. Fractions have been reported which differed from the original casein in solubility, phosphorus content, and reactions with rennet. Thus Linderstrøm-Lang (74) reported the separation of casein into fractions by means of alcohol containing a small quantity of hydrochloric acid. In general, his purified fractions were characterized by

differences in phosphorus content. Original casein containing 0.79% phosphorus was divided into fractions containing 0.10, 0.52, and 0.96% phosphorus, respectively, and accounting for 3, 25, and 68% of the total casein.

Mellander (86) found that casein was composed of three electrophoretic components, which he designated α, β, and γ in the order of decreasing mobility. The α- and γ-casein fractions were isolated in the electrophoresis apparatus. On the assumption that the caseins had 15.6% nitrogen, the phosphorus contents of α-casein and γ-casein were calculated as 0.96 and 0.05%. γ-casein had a low mobility at pH 6.98 in phosphate buffer. This fact later suggested to Mellander (87) that the electrophoretic diagram attributed to γ-casein was due to boundary anomalies. The electrophoretic experiments reported by Warner (140) showed a slow-moving component in the impure β-casein fraction. It seems likely that the γ-casein of Mellander is similar to the alcohol-soluble, low-phosphorus-containing casein isolated by Osborne and Wakeman (97), and by Linderstrøm-Lang (74). The amount of low-phosphorus protein in unfractionated casein appears not to be more than 5% of the total. Electrophoretic analysis of the low-phosphorus casein obtained by alcohol–water extraction is necessary to prove the identity of these proteins.

c. *Separation of α- and β-Caseins.* Warner (140) has applied electrophoretic analysis to the chemical fractionation of casein. He was unable to demonstrate a separation of the components by precipitating casein in the presence of salt followed by the use of acetone, as suggested in the work of Cherbuliez and Meyer (19), and Cherbuliez and Schneider (20). The method of Groh *et al.* (42) of precipitating from urea solution with alcohol produced some fractionation, though some of the fractions showed patterns not found in the electrophoretic pattern of the original casein. Some separation of the components was obtained by Linderstrøm-Lang's (74) method of fractionation with sodium chloride on the acid side of the isoelectric point. This procedure was inadequate for a complete separation with a reasonable number of precipitations. Warner separated α- from β-casein by taking advantage of the higher solubility of β-casein at pH 4.2.

Preparation of α-Casein. Warner prepared α-casein by dissolving undried, purified, acid-precipitated casein in sufficient sodium hydroxide to give a 1% solution with a pH of 6.5. The solution was made to pH 3.5 by adding dilute hydrochloric acid. After the solution was chilled to 2°, it was diluted to a concentration of 0.2 to 0.3% protein, and 0.01 N sodium hydroxide was added until pH 4.2 was reached. At this stage a precipitate formed, and the addition of sodium hydroxide was continued

until a clear supernatant was obtained on centrifugation. Usually this occurred at a pII 4.4. The precipitate was dissolved, and the entire procedure repeated at least six times. The isolated protein then showed an electrophoretic pattern of α-casein free of β-casein. Mellander (87) has used this method of separating α-casein from cow's casein, but he was unable to separate the α-component from human casein by this procedure.

Preparation of β-Casein. Warner obtained β-casein from the filtrates of α-casein fractions by warming to room temperature and adjusting the pII to 4.9. The protein precipitated under these conditions was largely β-casein. It was further purified by dissolving in sodium hydroxide to give a 0.2% solution. The solution was chilled to 2°, and 0.01 N hydro-

TABLE III
Composition of Casein Fractions

Constituent	α-Casein,[a] %	β-Casein,[a] %	Alcohol-soluble casein, % (97)
Phosphorus	0.99[b] (140)	0.60 (140)	0.06
Total N	15.5 (140)	15.4 (140)	15.6
Amino N	0.99 (37)	0.73 (37)	...
Arginine	4.3 (37)	3.4 (37)	2.9
Histidine	2.9 (37)	3.1 (37)	2.3
Lysine	8.9 (37)	6.6 (37)	4.0
Tyrosine	8.1 (37)	3.2 (37)	2.5
Tryptophan	1.6 (37)	0.6 (37)	...

[a] Reference numbers are given in parentheses.
[b] 0.97% phosphorus for α-casein separated by electrophoresis (86).

chloric acid was added until pII 4.9 was reached. The precipitate was filtered off and dissolved with sodium hydroxide to give a 0.03% protein solution. This solution was made to pII 4.5 at 2°. The precipitate formed (chiefly α-casein) was removed, and the filtrate was warmed to room temperature. The precipitate then formed was largely β-casein. A final purification was accomplished in the same manner by precipitating a 0.5% solution of the β-casein at 2° and pII 4.9.

d. Properties and Composition of α- and β-Caseins. These two fractions separated by Warner were not homogeneous over the entire pH range but were distinct fractions, neither one of which was contaminated with the other. The mobility of the purified α-casein in Veronal buffer at an ionic strength of 0.1 and a pII of 7.78 was 6.98 (cm.^{2} volt^{-1} sec.$^{-1}$ $\times$ 10^{-5}). However, in the presence of 20% β-casein, the mobility of the α-casein was reduced to 6.30 $\times$ 10^{-5}, indicating interaction of these two fractions. The mobility of purified β-casein was 3.27 $\times$ 10^{-5}, and its

mobility was only slightly changed by the presence of α-casein. Table III shows the composition of purified casein fractions.

e. Physical Properties of Casein. Since electrophoretic data indicate that casein is heterogeneous, it is not surprising that the reported molecular weights of casein should be unsatisfactory. It has been reported (133) that small amounts of calcium produce an extensive change in the sedimentation constant of casein. This may help explain the wide differences in the values reported for the molecular weight of this substance. Svedberg, Carpenter, and Carpenter (131) reported that casein prepared by the Van Slyke and Baker method is polydisperse, the main component having a molecular weight of 75,000 to 100,000 and a sedimentation constant of $S_{20} = 5.6 \times 10^{-13}$. Similar results were obtained with casein prepared by the Hammarsten method. The sedimentation constant of casein prepared in different ways was reported by Pedersen (99) to be 12×10^{-13} in phosphate buffers. In the experiments of Svedberg, Carpenter, and Carpenter, the salt concentration was only 0.017 M, whereas in Pedersen's experiments, the salt concentration was 0.25 M. This may explain the wide variations for the sedimentation constant of casein reported in these investigations. Burk and Greenberg (16) have reported a molecular weight of 33,000 for casein in 6.66 M solutions of urea.

The combination of calcium ions with casein has been investigated by means of the ultracentrifuge (18). The law of mass action accurately described the data, yielding the dissociation constant of calcium caseinate. The dissociation constant is independent of the concentration of protein and the pH from 6.3 to 8.5. Equations have been reported by Klotz (67) for describing the combination of casein with calcium ions in terms of sixteen dissociation constants rather than one.

Studies on flow birefringence and viscometry of sodium caseinate solutions by Nitschmann and Guggisberg (93) led to the conclusion that casein particles are rod-shaped, with an axial ratio of $a/b = 8.7$ and a particle length of 290 Å units, depending somewhat on the method of calculating. Data were also reported for streaming birefringence of sodium caseinate in 1.6 M sodium sulfate. Apparently an aggregation of casein molecules takes place in this salt solution, since Edsall (30) has calculated a particle length of 2200 Å units from these data.

In the separation of the components of casein, it is important to know whether decomposition has occurred during fractionation. It was noted by Cohn and Berggren (22) that casein solutions hydrolyze even in weakly alkaline solutions, with an increase in base-combining capacity and in water-soluble protein. It has often been observed that the viscosity of casein solutions decreases with time. That the change in viscosity is

enzymatic and is associated with an increase in nonprotein nitrogen was clearly demonstrated by Warner and Polis (141). No method for removing the proteolytic component from the bulk of the casein was found, though it was destroyed by heating casein solutions to 80°C. for 10 minutes at pH 8.6. To prove that the isolated components of casein were not the result of decomposition, Linderstrøm-Lang (74) and Warner (140) have compared the properties of the reconstituted casein with the unfractionated material. The reproduction of the electrophoretic pattern of the original casein by reconstitution with the separated components is strong evidence that the fractions were not changed during separation.

f. Phosphopeptones from Casein. Posternak (103) isolated phosphopeptone from tryptic digests of cow's casein. His first compound contained 5.9% phosphorus and 11.9% nitrogen and showed the presence of glutamic and aspartic acids, serine, and isoleucine. Since serine was the only hydroxy acid present, he considered it likely that phosphoric acid was bound to the hydroxy group of serine. Lipmann (77) isolated phosphoserine from casein, and Levene and Hill (71) isolated phosphoserylglutamic acid from phosphopeptone prepared according to Posternak. Independently, Rimington and Kay (107) described the preparation of a phosphopeptone by tryptic digestion of casein solutions. Their preparation contained 7.05% phosphorus and 10.13% nitrogen. Damodaran and Ramachandran (26) digested casein with pepsin followed by trypsin and isolated the barium salt of a phosphopeptone which contained 4.34% phosphorus and 6.46% nitrogen. This phosphopeptone was reported to be composed of three glutamic, three isoleucine, and four serine residues. Mellander (87), using the method of Damodaran and Ramachandran, has prepared phosphopeptone from human casein and cow's casein. He found approximately 50% of the total phosphorus of casein in the phosphopeptone. The presence of alkaline phosphatase in commercial trypsin reduced the yield of phosphopeptone. The amount of phosphatase in the trypsin was reduced by heating its solutions to 50°C. for 30 minutes at pH 2.0. As prepared by Mellander, the barium salt of the phosphopeptone from human casein contained approximately 6.3% nitrogen and 4.9% phosphorus, while that from cow's casein contained about 7.0% nitrogen 5.3% phosphorus. The large variations in composition among individual preparations indicated that the product probably was a mixture or that each preparation differed in structure. After a second treatment with trypsin and fractional precipitation, Nicolet and Shinn (92) isolated a phosphopeptone from casein containing eight amino acids. The peptide contained one molecule of serine, two of phosphoserine, two of isoleucine, and two of glutamic acid. The dipeptide phosphoserylglutamic acid was isolated and its structure determined.

g. Rennet Casein. Although the clotting of milk or casein by rennet has been much studied, the nature of the change produced in casein by this enzyme is unknown. It has not been possible to distinguish calcium-free preparations of rennet casein from acid casein by conventional analytical procedures. Since rennet casein cannot be clotted a second time, it would appear that there is a structural difference between acid and rennet casein. Many investigators have been concerned with the removal of the proteolytic action from rennet preparations. Berridge (9) has crystallized rennet and found that the pure enzyme has proteolytic activity but that the optimum pH for rennet proteolysis is more alkaline than the optimum for pepsin. Holter (53) considered the proteolytic property to be unimportant in coagulation of casein by rennet. Nitschmann and Lehmann (94) have recently reported that the action of rennet on casein solutions produces a change in the electrophoretic pattern of the casein. They incubated a 6% solution of sodium caseinate with rennet at 35°C. for 30 minutes at pH 6.6. After equilibrating with Veronal buffer at pH 7.4, which presumably destroyed rennet activity, an electrophoretic analysis of the solution was made. It was found that the electrophoretic pattern of rennet-treated casein differed from that given by the same casein solution treated with boiled rennet, in that the α-casein was split into two peaks. The mobilities of the α_1- and α_2-components were reported to be 9.55×10^{-5} and 8.83×10^{-5}, respectively, as compared with acid α-casein of 9.30×10^{-5}. The descending boundary of rennet-treated casein was not split into two peaks. Warner (140) found that the electrophoretic pattern of α-casein splits into two peaks in some preparations. In view of this, Nitschmann and Lehmann consider the action of rennet on α-casein as an acceleration of the tendency to split into two components. The possibility that other components of casein stabilize α-casein was also considered. These authors (95) have also compared the behavior of mixtures of rennet and acid casein with calcium chloride solutions. One of the two caseins was dyed by coupling with a diazonium salt, and the amount precipitated by calcium chloride from mixtures was determined colorimetrically. In this manner the relative amounts of acid and rennet caseins present were estimated. It was shown that from mixed solutions of rennet and acid caseins about equal amounts of rennet and acid caseins were precipitated by adding calcium chloride. This result is in agreement with the theory of Linderstrøm-Lang (74) that the stability of the calcium caseinate system is due to the protective colloid action of one of the components. Nitschmann and Lehmann attempted to estimate the percentage of the protective colloid fraction in acid casein and concluded that it was less than 20% of the total casein.

IV. Proteins of Whey

After casein is removed by isoelectric precipitation at pII 4.6, the whey contains between 0.6 and 0.7% protein or about 20% of the total protein of skim milk. The number of components and relative amounts of each, as shown by electrophoresis, are given in Table II. It has been recognized for a long time that whey contains a globulin fraction and an albumin fraction (113), as characterized by salt fractionation methods.

1. Globulin Fraction

Since the work of Sebelien (113) was reported, the classical globulin of milk has been prepared by saturating whey with magnesium sulfate. Crowther and Raistrick (25) used anhydrous magnesium sulfate for precipitating milk globulin. They observed that, like serum globulin, milk globulin could be separated into euglobulin and pseudoglobulin by dialysis. Smith (118) has found that globulin prepared from whey by repeated precipitation with saturated magnesium sulfate gave preparations which show complex electrophoretic patterns. He devised a method of preparing an electrophoretically homogeneous globulin with a mobility of 1.7 to 2.5 u by fractionation with ammonium sulfate. The crude globulin fraction was precipitated by 0.5 saturation with ammonium sulfate. The precipitate was dissolved to about 3% protein, the pII adjusted to 4.6, and ammonium sulfate added to 0.25 saturation. The precipitate was removed and discarded. The immune proteins were precipitated from the supernatant at 0.4 saturation with ammonium sulfate at pII 6.0. The precipitate was reworked by dissolving it in water at 1°, adjusting to pII 4.5 and removing an insoluble residue that formed. The supernatant was precipitated at 0.3 saturation with ammonium sulfate. The filtrate was made to pII 6.0 and ammonium sulfate added to 0.4 saturation. Both fractions appeared to be electrophoretically homogeneous. On dialysis, however, the euglobulin and pseudoglobulin separated from the final precipitate at 0.4 saturation appeared to be more homogeneous by electrophoresis than the corresponding fractions obtained at 0.3 saturation. As shown in Table II, the electrophoretic component of whey with a mobility of $u = -3.6$, which amounted to 18% of the protein of whey, appears not to have been separated in the pure form.

a. Colostrum Globulin. The marked difference in the physical and biological properties of colostral milk or colostrum obtained for the first few days after parturition can be ascribed primarily to its high protein content and atypical protein distribution. The total protein concen-

tration of cow's colostrum at birth may be about 17.5% (32). Of this, 5% is casein and 11.3% albumin and globulin. This high protein level falls rapidly in the first 24 hours and then gradually approaches the normal composition of milk, that is 3.7% total protein, of which 3.0% is casein and 0.7% is albumin and globulin. The most striking difference between colostrum and normal milk is the high globulin content of the colostrum.

By salt fractionation studies Howe (54–58) demonstrated the absence of euglobulin and pseudoglobulin I in the blood of a newborn calf. When colostrum was fed before the calf was 21 hours old, these globulins always appeared in the blood. When the calf was fed only milk, euglobulin and pseudoglobulin were absent from the blood for some time. Howe demonstrated the presence of euglobulin, pseudoglobulin I, and pseudoglobulin II in colostrum. He then showed that the high concentration of euglobulin and pseudoglobulin appearing in the blood of a calf after colostrum ingestion was transient. After the first day the concentration of these two proteins decreased, and the high levels characteristic of the adult animal did not appear until the age of 12 or 14 months. Associated with the feeding of colostrum (125), there was invariably an albuminuria. Howe (56) identified the excreted proteins as euglobulin and pseudoglobulin. Similar studies on the human subject were made by Lewis and Wells (72) and by Boyd (11). They found that the blood of an infant had practically no euglobulin, although it contained the same concentrations of pseudoglobulin I and II as the adult. This fraction gradually appeared in infants even when they were given no colostrum. It appeared much more rapidly, however, when colostrum was fed. It is apparent that the colostrum contains proteins with antibody activity that may be absorbed unaltered from the digestive tract of the young animal.

The importance of colostrum from the calf was indicated more directly by Smith and Little (124), who found that more calves fed colostrum survived than those deprived of colostrum and fed milk. They concluded that colostrum protects the young animal against organisms which are harmless to it later when its own anti-infective facilities are in operation. About the same time Orcutt and Howe (96) showed the passage of natural agglutinins of *Brucella abortus* from a cow to her calf by the globulins of the colostrum. Timmerman (137) reported the presence of agglutinins for typhoid bacillus in human colostrum during the first five days of suckling. Kuttner and Ratner (70), however, indicated that colostral antibodies do not have the same importance for humans as for animals. They found the blood of infants born from mothers who were not immune to diphtheria to contain no antitoxin. The blood of infants born to immune mothers had as much antitoxin as the mother's blood

even before they received colostrum. They infer, therefore, that the important mode of transfer of antibody from the human mother to the infant is by way of the placenta. Jameson *et al.* (60) found that feeding cow's colostrum to man and to the adult rat produced an additional globulin component in the serum. The α-fraction was greatly increased, and the γ-fraction was either split or a new fraction appeared. Highly immune rat serum showed similar changes. The feeding of other proteins like casein, serum albumin, serum globulin, and liver and kidney protein did not have this effect.

Early work on the chemical composition of colostrum showed the presence of the three main protein components of milk. Crowther and Raistrick (25) did a comparative study of the proteins of the colostrum and milk of the cow and their relations to serum proteins. With the methods available to them at the time (1916) they arrived at the conclusions: (*a*) that casein, total lactoglobulin, and lactalbumin are distinct proteins and have the same composition whether prepared from colostrum or normal milk; (*b*) the globulin fractions obtained from colostrum and milk, although occurring in small amounts in milk, are alike and closely allied to or identical with the serum globulin from ox blood; (*c*) the eulactoglobulin and pseudolactoglobulin are identical insofar as composition is concerned; and (*d*) the lactalbumin from colostrum or milk is different from blood serum albumin. Woodman (145) by the method of protein racemization, with which he claimed the ability to establish the identity or nonidentity of related proteins, arrived at similar conclusions.

An outstanding and definitive study of the proteins of colostrum was made recently by Smith *et al.* (117,119–123). Whole colostrum and the various fractions isolated by conventional precipitation procedures with ammonium sulfate were studied electrophoretically. Table IV, taken from Smith's data, illustrates in concise manner the colostrum protein picture. Fraction A, the casein, was obtained by isoelectric precipitation at pH 4.5. It is obvious that this complex protein fraction does not have the distribution found in normal milk. This has been proved in greater detail by other studies (80) on the variation of the casein composition with the stage of lactation. Fraction B was obtained between 0 and 0.3 saturation, with ammonium sulfate, and fraction C was obtained between 0.3 and 0.5 saturation. These two fractions contain most of the colostral protein. By precipitation at 0.4 saturation with ammonium sulfate at pH 6.0, followed by solution and reprecipitation, Smith was able to isolate an electrophoretically pure globulin from colostrum in high yield. Apparently this procedure is not applicable to the isolation of the globulin from normal whey. Probably because of the preponderance of this protein in colostrum, Smith was able to obtain it quantitatively free of other pro-

teins, when determined electrophoretically. This protein fraction completely accounts for all the immune properties of the colostrum.

The immune lactoglobulin isolated in electrophoretically pure form was subjected to more extensive investigation of its properties. By exhaustive dialysis, the immune lactoglobulin could be separated into water-insoluble euglobulin and water-soluble pseudoglobulin fractions. The euglobulin migrated in Veronal buffer, pH 8.5, with a velocity of $-1.9\,u$, as compared with $-2.2\,u$ for the pseudoglobulin. Both fractions

TABLE IV
Electrophoretic Composition of Colostrum and Fractions from Colostrum[a]

Fraction	Dry wt. isolated, g.	u	%	u[b]	%	u	%	u	%	u	%	u	%
Whole colostrum	355	...		−1.8	54	−2.6	7	−4.2	35	−5.5	2	−7.2	2
(A) Casein	60	−1.3	1	−1.8	1	−2.7	6	−4.0	18	−5.1	74		
(B) Immune lactoglobulin	101	...		−2.1	100	...		...		...		...	
(C) Immune lactoglobulin	113	...		−2.1	85	−3.6	5	−4.6	10	...		...	
(D) β-Lacto-globulin	17	...		−2.2	4	−3.2	5	−4.3	75	−5.8	10	−6.3	4
												higher	2

[a] From the study of Smith (117). Electrophoresis patterns were obtained with Tiselius apparatus at 1°C. in Veronal buffer, pH 8.3 to 8.4, ionic strength 0.1.
[b] Immune lactoglobulin.

had immune activity. The total colostral globulin had an isoelectric point at pH 5.85. The euglobulin from another animal was isoelectric at pH 6.2, the pseudoglobulin at pH 6.0. With respect to the variations of mobility with pH, the colostrum globulin resembled the plasma T-globulin more than the γ-globulin. The immune globulin from colostrum always contained carbohydrate. Smith et al. (123) reported 2.65% of the carbohydrate as hexose and 1.48% as hexosamine. The molecular weight of immune lactoglobulin was reported at about 160,000 to 190,000. The diffusion constant D_{20} was given as 3.6×10^{-7} (117). Amino acid values for colostrum globulin as reported by Smith and Greene (117,121) and by Hansen et al. (48) are given in Table V (see p. 218).

The colostrum immune globulin and plasma γ-globulin were quantitatively equivalent in producing anaphylaxis in guinea pigs. The immune activity of bovine plasma is present in both T- and γ-components. On the basis of comparison of the elementary composition, isoelectric points, diffusion constants, and amino acid analyses of colostrum globulin

with those of T- and γ-globulin, Smith (121) concludes that, although these three proteins, which are associated with immune activity in the cow, are closely related, they are not identical.

2. "Albumin" Fraction; β-Lactoglobulin

The portion of whey soluble in saturated magnesium sulfate or 0.5 saturated ammonium sulfate is commonly designated the albumin fraction. This fraction contains a variety of proteins, some of which have enzymatic properties. Wichmann in 1899 (143) crystallized lactalbumin from this fraction with ammonium sulfate. Later Sjögren and Svedberg (116) also obtained lactalbumin in crystalline form from whey with ammonium sulfate and dilute sulfuric acid. Palmer (98) was unable to obtain crystalline lactalbumin by this method. Sørensen and Sørensen (127) also had difficulty in preparing lactalbumin. Palmer (98), however, prepared a crystalline protein from the albumin fraction of whey in good yield by prolonged dialysis of the proteins of this fraction at pH 5.2 in the absence of salt. This crystalline protein has been named β-lactoglobulin (17), since it was identified with the β-component of milk serum, as shown by ultracentrifugal studies (99). Svedberg and Pedersen (132) are of the opinion that the previously described crystalline lactalbumin represents an impure β-lactoglobulin. The view that crystalline β-lactoglobulin is essentially the same as crystalline lactalbumin appears probable, since Sørensen and Sørensen (127) had no difficulty in recrystallizing β-lactoglobulin with ammonium sulfate at pH 6-7. They also described (127) a crude crystalline lactalbumin preparation which was not sufficiently characterized to determine whether it differed from crystalline β-lactoglobulin. In addition, they described the separation of lactomucin, a green mucoid fraction, a red fraction, and a crystalline protein insoluble in water and dilute salt solutions. The latter protein was crystallized from aqueous solutions at pH 6.5 to 7.0 by the addition of ammonium sulfate. Pedersen (99) has attributed a component of whey with a sedimentation constant $S_{20} = 1.9 \times 10^{-13}$ to a lactalbumin isolated by Kekwick (unpublished).

a. Preparation of β-Lactoglobulin. In Palmer's (98) first method for preparing β-lactoglobulin, casein was precipitated by adding hydrochloric acid to pH 4.6. The pH of the whey was then made 6.0, and the solution half saturated with ammonium sulfate to remove the globulin. The filtrate then was saturated with ammonium sulfate, and the precipitated albumin redissolved in water. On long dialysis at pH 5.2, an oil accumulated, which gradually changed into large crystals. Palmer also described a modification of this method which consists in concentration of whey by freezing, followed by fractionation with sodium sulfate. He obtained a

yield of 1.8 g. of crystalline β-lactoglobulin per liter of whey or 60% of the total protein in the albumin fraction. The first method of Palmer, which involves the use of ammonium sulfate, is convenient when large amounts of β-lactoglobulin are to be prepared. Sørensen and Sørensen's (127) method consists in precipitation of casein and the globulin fraction with ammonium sulfate. β-Lactoglobulin is obtained from the portion of whey soluble in 2.3 M but insoluble in 3.3 M ammonium sulfate. In recrystallizing β-lactoglobulin, Palmer used sodium chloride or dilute ammonium hydroxide for dissolving the β-lactoglobulin. It has been reported (15) that dissolving β-lactoglobulin in dilute sodium hydroxide produces a change in the molecular weight, as determined by osmotic pressure. Separating β-lactoglobulin by means of alcohol at low temperatures has been suggested (4).

b. Properties of β-Lactoglobulin. Palmer (98) reported the solubility of β-lactoglobulin in water, dilute sodium chloride, and concentrated sodium sulfate solutions. He observed that a fourfold increase in the amount of solid phase did not change the solubility, indicating the relative purity of this protein. The effect of varying the amount of solid phase on its solubility in dilute ammonium chloride solutions was extensively investigated by Sørensen and Palmer (126). They found that the change in solubility with a threefold variation in the amount of solid phase was not more than 1%. These results on its solubility as well as the elegance of the crystal form and other properties of β-lactoglobulin have made it a favorite protein for investigation. Pedersen (100) calculated the molecular weight to be 39,000, and found it to be stable from pH 1 to 9. From the manner in which the sedimentation and diffusion constants varied with the pH of the solution, he assumed that the molecular weight remained constant and that the shape of the molecule changed. He found the isoelectric point to be pH 5.19 in acetate buffers by the electrophoretic method. Crowfoot (24) determined its crystal structure and unit cell by means of X-ray measurements. Assuming 8 molecules per unit cell, she calculated a molecular weight of 40,000 for air-dried β-lactoglobulin (33). A value of 35,800 for the anhydrous molecular weight of β-lactoglobulin was deduced from Crowfoot's data on the assumption that the air-dried crystals contained 9.78% moisture (83). A recent X-ray investigation of this protein has given a value of 35,000 for the anhydrous molecular weight (114). A value of 35,050 for the molecular weight of β-lactoglobulin has been found by osmotic pressure measurements in 0.5 M sodium chloride (15). McMeekin and Warner (83) determined directly the amount of water in a single β-lactoglobulin crystal and found it to be 46% of the total weight. It was also shown that ammonium sulfate penetrated the crystal and, on the assumption that the

salt dissolved in the water of crystallization, the concentration of salt in the water of crystallization reached 82% of the salt concentration in the surrounding liquid. The dissociation curve of β-lactoglobulin has been determined under a variety of conditions (17). The effects of temperature, concentration of potassium chloride, concentration of protein, and the addition of formaldehyde on the dissociation curve were evaluated. The results were consistent with the presence of 58 carboxyl, 34 amino, 6 imidazole, and 6 guanidino groups in 1 mole (40,000 grams) of β-lactoglobulin. The ratio of the net charge to the electrophoretic mobility was constant from pH 4 to 9. A complete amino acid analysis of β-lactoglobulin has been reported by Brand et al. (13). Their results are tabulated in Table V with minor modifications. The amino acid composition of β-lactoglobulin differs markedly from the amino acid composition of serum albumin. This is consistent with the differences noted by earlier workers between the composition of lactalbumin and serum albumin.

Grönwall (41) has reported extensive solubility studies on β-lactoglobulin under a variety of conditions. Certain aspects of this work have been reviewed by Edsall (31). In agreement with Palmer's results, it was found that the solubility did not increase more than 1% when the protein nitrogen in the solid phase was increased several times. As a test for purity, these results as well as those of Palmer are deficient in two respects, namely, (a) the amount of solid phase is much too great to demonstrate purity, being from five to ten times greater than the solubility, and (b) there is no indication that the values for solubility are equilibrium values, since the solubility was determined after a period of 24 hours only. A value of 0.16 mg. of nitrogen per milliliter was found for the solubility of β-lactoglobulin in water, which is somewhat lower than the 0.19 reported by Palmer. The value for the solubility in water obtained by extrapolating the salt solubility curve to zero salt concentration was 0.14 mg. of nitrogen per milliliter. Grönwall found that the solubility in sodium chloride solutions varied from one preparation to another, even though the preparations were made by identical methods. His results for the solubility in dilute sodium chloride solutions were twice as great as those found by Palmer. These solubility results, as well as the electrophoretic results of Li, indicate that β-lactoglobulin is not a homogeneous protein. Li (73) found that β-lactoglobulin contained three components as determined by electrophoresis at pH 4.8 and 6.5. Pedersen (100) had previously reported β-lactoglobulin to be electrophoretically homogeneous when a 0.2% protein solution was used. Bosshardt, Moore, and Brand (10) found freshly crystallized β-lactoglobulin to be electrophoretically homogeneous at pH 4.0, 7.4, and 8.6. By implication, this report suggests that the heterogeneity of β-lactoglobulin is produced by recrystal-

lization.   Variations in the solubility of β-lactoglobulin in water and sodium chloride solutions have been correlated (82) with variations in its electrophoretic pattern (79). β-Lactoglobulin preparations with different electrophoretic composition were obtained by fractionation with acetate buffers and also with alcohol.   These preparations gave different solubilities in water and sodium chloride solutions.

Further β-lactoglobulin fractions of different properties were separated from whey proteins after the partial removal of β-lactoglobulin by the method of Palmer.   These preparations varied in solubility and in electrophoretic composition at pH 4.8, but were homogeneous at pH 8.4.   Since the dielectric increment is large—1.5 units per gram per liter (34)—it is to be expected that the solubility of β-lactoglobulin will be markedly influenced by ions and dipolar ions, making complete separation from other proteins difficult.   Grönwall has demonstrated that the solubility of β-lactoglobulin is increased in solutions of glycine and glycine peptides proportionally to the dielectric constant of the solvent.   The effect of ovalbumin in increasing the solubility of β-lactoglobulin was of the order to be expected from the influence of ovalbumin on the dielectric constant of the solvent.

Davis and Dubos (27) have reported that β-lactoglobulin binds fatty acids in a manner similar to the binding by serum albumin.   A crystalline compound of β-lactoglobulin with two equivalents of dodecyl sulfate has been described (81).   This derivative is apparently undissociated, since the dodecyl sulfate was not removed by barium ion.   The mobility of the derivative was about 8% greater than that of β-lactoglobulin at pH 8.4, and the apparent isoelectric point was slightly more acid than that of β-lactoglobulin.   The solubility of the derivative is about one-half as large in water and one-third as large in dilute salt solution as that of β-lactoglobulin.   The derivative has a greater alkali-combining capacity than β-lactoglobulin, but has essentially the same acid-combining capacity.   Solutions of the derivative require a higher temperature for heat coagulation than do β-lactoglobulin solutions.   This effect is similar to the influence of dodecyl sulfate on the heat coagulation of albumin solutions (6).

Several studies have been made on the denaturation of β-lactoglobulin (14,59,75).   Briggs and Hull (14) demonstrated that the heat denaturation of β-lactoglobulin at pH 7.0 in a buffer solution of 0.1 ionic strength involves at least two reactions which could be followed by electrophoretic mobilities.   The first process, initiated only above 65°, was accompanied by a fourfold increase in particle weight and an increase in frictional ratio. The second process, which takes place only after the first process has occurred, was accompanied by a marked increase in electrophoretic

TABLE V

Amino Acid Composition of Milk Proteins[a,b]

Constituent	Human casein	Cow casein	Cow β-lacto-globulin	Cow colostrum globulin	Immune globulin of cow milk
Total N	15.1 (87)	15.65 (84)	15.60 (13)	15.63 (117)	15.67[c] (118)
Total S	0.78 (102)	0.78 (61)	1.60 (13)	1.1 (48)	1.00[c] (121)
Amino N		0.93 (88)	1.24 (13)		
Amide N		1.42 (104)	1.07 (13)		
Glycine	0.0 (144)	1.9 (115)	1.4 (13)		
Alanine	2.0 (144)	3.5 (138)	7.4 (66)		
Valine	5.0 (144)	7.2 (51)	5.8 (13)	10.2 (117)	9.6 (123)
Leucine	12.2 (144)	10.3 (51)	15.6 (13)	8.9 (117)	9.6 (123)
Isoleucine	6.3 (144)	7.6 (51)	6.1 (122)	2.77 (121)	3.03[c] (121)
Proline	8.9 (144)	11.6 (51)	4.1 (13)	10.0 (48)	
Phenylalanine	5.8 (144)	5.5 (51)	3.5 (13)	3.6 (117)	3.88 (123)
Cysteine			1.11 (13)		
Half-Cystine	0.6 (144)	0.34 (61)	2.29 (13)	3.26 (121)	3.15[c] (121)
Methionine	2.70 (102)	3.1 (61)	3.22 (13)	0.89 (121)	0.90[c] (121)
Tryptophan	1.05 (102)	1.2 (129)	1.94 (13)	2.74 (117)	2.70 (123)
Arginine	3.58 (102)	4.0 (84)	2.88 (13)	4.36 (121)	4.05[c] (121)
Histidine	2.0 (144)	3.2 (84)	1.58 (13)	1.99 (121)	2.05[c] (121)
Lysine	5.6 (144)	8.2 (35)	11.4 (13)	6.3 (121)	6.8[c] (121)
Aspartic Acid	4.6 (144)	7.2 (45)	11.4 (13)	9.4 (48)	
Glutamic Acid	20.9 (144)	22.0 (3)	19.5 (13)	12.3 (48)	
Serine	5.4 (144)	5.9 (104)	5.0 (13)		
Threonine	4.5 (144)	4.5 (104)	5.85 (13)	9.4 (121)	10.5[c] (121)
Tyrosine	6.11 (8)	6.1 (78)	3.78 (13)	6.7 (48)	
Totals	*97.24*	*113.34*	*113.85*	*92.81*	*56.26*

[a] Grams per 100 grams protein.

[b] Reference numbers are given in parentheses.

[c] Average of milk euglobulin and milk pseudoglobulin.

mobility and particle weight and followed the concentration time characteristics of a second-order reaction. The second reaction was repressed at 75°C. and did not take place at 99°.

The denaturation of β-lactoglobulin in urea solutions has been investigated by Jacobsen and Christensen (59). Denatured β-lactoglobulin was precipitated by the addition of 10 volumes of a mixture consisting of 0.8 M acetic acid, 0.4 M sodium acetate, and 0.5 M mag-

nesium sulfate. It was found that denaturation was fast at 0° and much slower at 37.4°. The protein denatured at 0° was rapidly reversed when the temperature was increased to 34.4°. The presumably denatured and reversed protein was recrystallized by adding saturated ammonium sulfate solution to two-thirds saturation.

V. Amino Acid Composition of Milk Proteins

The methods available for separating the electrophoretic components of casein are still inadequate. As a consequence, unfractionated acid-precipitated casein is a much studied and utilized protein. The results of recent amino acid analyses on both cow's and human unfractionated caseins are included in Table V with amino acid analyses of other milk proteins. The amino acid composition of human and cow's caseins appears to be much the same. This is particularly true when the results of a single investigation are compared (144). The phosphorus content of human casein is much lower than that of cow's casein. The sulfur content of human casein appears to vary widely, depending on the method of separation (87), but 0.78% has been selected for Table V.

VI. Enzymes in Milk

The presence of an enzyme in milk was first demonstrated by Arnold (2) in 1881. Since then, although a considerable number of oxidative and hydrolytic enzymes of milk have been reported, only two, xanthine oxidase (5) and lactoperoxidase (135), have been isolated and purified extensively. Any enzyme obtained from milk is not necessarily a true milk enzyme but may be derived from bacteria or leucocytes in the milk. The origin of milk enzymes is at best poorly defined. That they may be infiltrated plasma enzymes or secreted mammary gland enzymes are subjects more for a speculative than a factual report. The collection and isolation of the enzyme in the presence of bacteriostatic agents such as chloroform or formaldehyde and the isolation of the milk enzyme from the mammary gland itself have been cited as criteria for true milk enzymes.

1. Carbohydrases

a. Amylase. Of all the enzymes in normal milk, amylase is probably the least variable in quantity. Koning (68) reported the decomposition of 22.5 mg. of soluble starch in half an hour by 100 ml. of milk from healthy cows. Colostrum and milk from diseased udders show greater amylase activity than normal milk (21). Human milk has a higher amylase content than that of the cow or of other species (52,91,112,146). Cream has a higher amylase activity than skim milk. Most of the amylase is precipitated with the casein fraction of the milk proteins,

although some remains in the whey. Milk amylase shows maximum activity at pH 5.8 to 6.2 at 30°C. It is inactivated by heating for 1 hour at 60 to 65°C. (38,50). Because of the low amylase content of old milk, Giffhorn (36) proposed the use of amylase activity as a criterion of the quality of milk.

b. Lactase. This enzyme acts on lactose in milk splitting the molecule into glucose and galactose. Stoklasa (128) and Vandevelde (139) have reported that it is present in normal milk. This has been denied by Svanberg (130).

2. *Dehydrogenase*

a. Xanthine Oxidase. (This enzyme is also known as aldehydrase and Schardinger enzyme.) In 1902, Schardinger (111) reported that the addition of an aldehyde and methylene blue to fresh milk resulted in the disappearance of the blue color in the absence of oxygen. Later Morgan, Stewart, and Hopkins (90) and Dixon and Thurlow (29) demonstrated that milk contains an enzyme which oxidizes xanthine to uric acid. As shown by Ball (5), the enzyme is adsorbed on the fat globules in milk. It can be readily prepared by extracting the cream with disodium acid phosphate at 38°C. Purification is accomplished by digestion with commercial lipase, clarification with calcium chloride, and precipitation by saturation with ammonium sulfate to 60% at 0°C. The enzyme can be further purified by precipitation with ammonium sulfate to 33% saturation.

Corran *et al.* (23) have isolated a flavoprotein from milk which exhibits both xanthine aldehyde oxidase and dihydrocoenzyme I oxidase (diaphorase) activity. Their method of preparation involved fractionation of milk with ammonium sulfate, precipitation of the active fraction with alcohol, adsorption and elution of the enzyme from alumina gel and further concentration and fractionation with ammonium sulfate.

These milk flavoproteins catalyze the oxidation of xanthine, aldehyde, and dihydrocoenzyme I. Xanthine oxidase is not specific toward any one purine. It oxidizes a variety of aliphatic and aromatic aldehydes. When reacting with purines or aldehydes, it also can reduce nitrates to nitrites (29,44). Keilin and Hartree (63) have shown that if ethyl alcohol and catalase are added to a digest containing xanthine oxidase and hypoxanthine, the ethyl alcohol is oxidized to acetaldehyde. The flavin moiety of milk xanthine oxidase is similar to, if not identical with, flavin adenine dinucleotide (23). Ball (5) succeeded in the reversible resolution of milk flavoprotein by prolonged dialysis against distilled water at 0°C. Corran and coworkers (23) were unable to split the flavin reversibly from their preparation. Preparations of xanthine oxidase

dried or treated with cyanide are irreversibly inactivated. The dihydro-coenzyme I oxidase activity, however, is unaffected. Heating for 10 minutes at 80°C. denatures the protein and liberates the coenzyme (23). Xanthine oxidase has its isoelectric point at pH 6.2. Ball has calculated its maximum molecular weight to be 74,000. The absence of xanthine oxidase in human milk has been made the basis of a test for the distinction between human and cow's milk (108).

3. Esterases

a. Lipase. Employing high-fat creams, saturated with sucrose as a preservative, for a substrate, Rice and Markley (106) indicated definitely that a true lipase resembling pancreatic lipase is present in cow's milk. Hippius (52) and Moro (91) showed that human milk has a higher lipase content. The enzyme is inhibited by high acidity. It is destroyed by heat (20 minutes at 63°C.) and ultraviolet light (62), and is inactivated by traces of heavy metals, 0.2% fluoride, and 0.1% hydrogen peroxide. Homogenation of milk activates the lipase. There is a definite relationship between the oestrus cycle and lipase activity (65). The amount of enzyme usually increases with advanced lactation and with any abnormality of the udder. The addition of pitocin has been shown to activate the tributyrinase activity of milk (64).

b. Phosphatase. Raw milk contains a phosphatase that has the usual characteristics of mammalian phosphatase in its specific activation by magnesium ions and in its pH activity relations (39). The activity ranges from pH 6–10, with the optimum at pH 9. Massart and Vandendriessche (85) have shown that the phosphatase of milk is not inhibited by sodium fluoride. Inhibition is obtained by potassium cyanide and cysteine. The enzyme is activated both by zinc and magnesium. On the basis of inhibition and acceleration experiments on the activity of the enzyme, these investigators conclude that milk phosphatase is a metallo-protein and that zinc is the activating metal.

Guiltonneau and coworkers (43) have reported the presence of two phosphatases in cow's milk, as determined in butter and buttermilk. The enzymes are present in weak concentration in whole milk, are absent in completely skimmed milk, and are present in large amounts in buttermilk and the aqueous portion of butter. Because they find two zones of maximal activity, pH 4.2 and 7.6 to 7.8, the former maintaining activity after heating to 73°C. for 50 minutes while, in the latter zone, activity is destroyed after heating for 20 minutes at 63°, they conclude that two enzymes in milk will convert pyrophosphate to orthophosphate. Milk phosphatase is destroyed by heat but more slowly than bacterial organisms so that it is used as a test for pasteurization (85).

4. Protease

The presence of a presumably nonbacterial proteolytic enzyme in normal milk has been demonstrated by numerous investigators, notably by Thatcher and Dahlberg (134). The enzyme described is tryptic in nature; the proteins are broken down below the peptone stage Almost all the proteolytic activity in milk is precipitated with the casein when milk is acidified. Warner and Polis (141) showed the presence of a proteolytic enzyme in commercial and purified laboratory preparations of casein. The enzyme they studied had optimal activity at pH 9.2 in borax buffer, and Warner obtained a 150-fold increase in its activity by precipitation of a dialyzed casein enzyme digest at pH 4.5 and fractionation of the filtrate with ammonium sulfate. His concentrated enzyme showed four boundaries on electrophoresis, the proteolytic activity migrating with one of the two slower boundaries.

5. Oxidases

a. Catalase. That catalase in milk is a secreted enzyme has been shown by Grimmer (40) and Harden and Lane-Claypon (49). The catalase content of milk varies with breed (69), individual animal, time of milking, and feed. Colostral milk has a higher catalase content than normal milk (105). The enzyme is not uniformly present in human milk. Cream and separator slime contain higher proportions of the enzyme than the milk. Increased bacterial or leucocyte counts are invariably followed by higher catalase contents. The catalase activity is precipitated with the casein. The maximum activity of the enzyme at 0°C. occurs at pH 6.8 to 7.0 (7). The catalase activity of milk is completely destroyed by heating for 30 minutes at 65° to 70°C.

b. Lactoperoxidase. This enzyme has the distinction of being not only the first enzyme demonstrated in milk (2) but also the only milk enzyme reported isolated in a crystalline state (135). This was accomplished by fractionation with ammonium sulfate to remove the casein, heating to 70°C. for 15 minutes to remove inert protein, precipitation of other impurities with basic lead acetate, removal of red impurities with acetone, and final purification by electrophoresis. The yield does not exceed 0.2 g. from 100 liters of milk or about 2% of the total amount present in milk. The enzyme is a hemin protein that has the properties of an albumin. The lactoperoxidase shows distinct differences, as compared with Agner's (1) verdoperoxidase isolated from leucocytes.

Recrystallized lactoperoxidase was found to be homogeneous in a physicochemical respect by Theorell and Pedersen (136). These investigators reported the molecular weight to be 93,000, the sedimentation

constant $S_{20} = 5.37$, the diffusion constant $D_{20} = 5.95 \times 10^{-7}$, the partial specific volume $V = 0.764$, the frictional ratio $f/f_0 = 1.18$. The iron content, 0.07% was slightly higher than 1 mole of iron per mole of enzyme. Light absorption coefficients reported in the region 2400–6900 Å showed peaks at 600, 500, 410, and 280 mμ.

VII. Relationship of Milk Proteins to Serum Proteins

The proteins of milk have often been compared with the proteins of blood serum (25,47,48,118,142). The purity of the proteins has always been a complicating factor in such comparisons. It was early recognized that casein differed chemically and biologically from the proteins of serum (127,142). The immunological evidence of Wells and Osborne indicated that whey proteins are related to the serum proteins of the same animal. Their results demonstrated, however, that lactalbumin differs from serum albumin. This finding was in agreement with the dissimilar composition of these two substances (25). The relatively complete amino acid analysis of Brand et al. (12) on β-lactoglobulin and serum albumin also show that these proteins differ in composition. That the proteins of milk whey and of blood serum are characteristic for the species has been demonstrated by electrophoretic studies (28).

Smith's (119) comparison of the amino acid composition of the immune globulin of milk with that of the immune globulins of serum revealed a remarkably similar amino acid pattern, though individual differences were found. His mobility studies indicated a close similarity of the immune globulin of milk with the T-component of serum rather than with γ-globulin.

The component of whey in Table II with a mobility of $-6.4\ u$, amounting to 5% of the protein of whey, has a mobility close to that of serum albumin (110). Based on immunological results, Peskett (101) has reported that normal milk contains a small amount of serum albumin and that it increases during the secretion of abnormal milk.

ADDENDUM

Gordon et al. (147) have recently reported the results of complete amino acid analyses on whole casein, α-casein and β-casein. Their results are given in Table VI. These values for the amino acid contents of the caseins supplement the previously reported values in Table III.

The apparent specific volumes of whole casein, α-casein and β-casein, calculated from densities (148), are in excellent agreement with the specific volumes calculated from the amino acid residues as reported by Gordon et al.

TABLE VI
Amino Acid Composition of Caseins
g./100 g. protein

	Whole Casein	α-Casein	β-Casein
Total N	15.63	15.53	15.33
Total P	0.86	0.99	0.61
Amino N	0.93	0.99	0.72
Glycine	2.7	2.8	2.4
Alanine	3.0[a]	3.7[a]	1.7[a]
Valine	7.2	6.3	10.2
Leucine	9.2	7.9	11.6
Isoleucine	6.1	6.4	5.5
Proline	11.3	8.2	16.0
Phenylalanine	5.0	4.6	5.8
Cystine	0.34	0.43	0.0-0.1
Methionine	2.8	2.5	3.4
Tryptophan	1.2	1.6	0.65
Arginine	4.1	4.3	3.4
Histidine	3.1	2.9	3.1
Lysine	8.2	8.9	6.5
Aspartic acid	7.1	8.4	4.9
Glutamic acid	22.4	22.5	23.2
Amide N	1.6	1.6	1.6
Serine	6.3	6.3	6.8
Threonine	4.9	4.9	5.1
Tyrosine	6.3	8.1	3.2
Total	115.8[b]	115.7[b]	117.4[b]

[a] These values are provisional.
[b] Total includes amino acids, amide N calculated as ammonia, and phosphorus calculated as phosphoric acid.

TABLE VII
Apparent Specific Volumes of Caseins

	Apparent specific volume calculated from amino acid residues	Apparent specific volume from densities at 25°C.
Whole Casein	0.731	0.731
α-Casein	0.725	0.728
β-Casein	0.743	0.741

Table VII shows the results obtained by the two methods for calculating the apparent specific volumes.

The excellent agreement of the values by the two methods indicates that the apparent specific volume of a protein is essentially determined by the volume of its amino acid residues.

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